16S rRNA gene NCBI ID numbers from the PICO project

Website: https://www.bco-dmo.org/dataset/704695
Data Type: Other Field Results
Version: Final
Version Date: 2017-06-13

Project
» Pivers Island Coastal Observatory (PICO)
» Collaborative Research: Ocean Acidification: microbes as sentinels of adaptive responses to multiple stressors: contrasting estuarine and open ocean environments (OA microbe adaptation)

Program
» Science, Engineering and Education for Sustainability NSF-Wide Investment (SEES): Ocean Acidification (formerly CRI-OA) (SEES-OA)

Contributors	Affiliation	Role
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Coverage

Spatial Extent: **Lat**:34.7181 **Lon**:-76.6707

Dataset Description

Sequences for 16S rRNA gene (bacterioplankton) amplicon libraries for size-fractionated libraries including the large-particle-associated bacterial communities (>63um), small-particle-associated bacterial communities (63 to 5 um), ambiguous bacterial communities (e.g. either large cells or those attached to small particles; 5 to 1-um), and free-living bacterial communities (<1 um).

For related research, go to: http://oceanography.ml.duke.edu/johnson/research/pico/

Methods & Sampling

Seawater was collected at ~10:30 AM local time using a 5-L Niskin bottle centered at 1 m or a peristaltic pump with the tubing open at 1 m, and processed immediately. To characterize particle-associated and free-living

microbial communities, the large-particle-associated bacterial communities (>63um), small-particle-associated bacterial communities (63 to 5 um), ambiguous bacterial communities (5 to 1-um), and free-living bacterial communities (<1 um) were separated by sequential gravity filtration using a 63-um-pore-size plankton net followed by 5-um-pore-size (Advantec MFS), 1-um-pore-size (GE Water& Process Technologies), and 0.2-um-pore-size (Pall Supor-200) filters. Large particles collected on the 63-um-pore-size plankton net from 10 liters of seawater were resuspended in autoclaved artificial seawater and concentrated on 0.22-um-pore-size filters. Filtered volumes were chosen such that the filtration rate did not become visibly lower during collection, and filters were rinsed with autoclaved artificial seawater to remove additional material smaller than the filter pore size. All filters were stored at -80 deg C until DNA extraction.

DNA extraction and library preparation. We extracted DNA from the size-fractionated samples using a Puregene Yeast/Bacteria kit (Qiagen) according to the manufacturer's instructions supplemented with three rounds (60 s each) of bead beating. Microbial communities were characterized using a dual index sequencing approach (Kozich et al. 2013) with the following portions of the primers targeting the V3-to-V4 region of the bacterial and archaeal 16S rRNA genes: for 16S F V3, CCTACGGGNGGCWSCAG; and for 16S R V4, GGACTACNVGGGTWTCTAAT (Hugoni et al. 2013). PCR mixtures contained 0.4Uof Q5DNApolymerase (NEB) as well as a final concentration of 200µM deoxynucleoside triphosphates (dNTPs), 2mM $MgCl_2$, and 0.5 uM primers. PCRs were performed using thermocycling with the following protocol: 98 deg C for 30 s followed by 35 cycles at 98 deg C for 10 s, 55 deg C for 30 s, and 72 deg C for 30 s, with a final extension at 72 deg C for 2 min. Triplicate reaction mixtures per sample were pooled and gel purified**.**

DNA extraction and sequencing

Microbial biomass was collected by filtering ~1 L of seawater through a 0.22-micron Sterivex filter (Millipore) and filters were stored at -80 deg C until extraction. Nucleic acids were extracted as described previously (Massana et al 1997), with some modifications. In brief, cells were lysed by bead-beating on ice three times for 30 secs in lysis solution (0.75 M sucrose, 40 mM EDTA, 50 mM Tris pH 8.0), followed by consecutive incubations with lysozyme (60 mg/mL; 37 deg C) and SDS (1%; 55 deg C). DNA was purified by phenol-chloroform extraction, RNase treatment, isopropanol precipitation, and PCR inhibitor removal (Zymo). DNA concentration was measured using a NanoDrop ND-1000.

Microbial communities were characterized using a dual index amplicon library approach targeting the 16S rRNA gene V3-V4 region (Kozich et al 2013, Yung et al 2016). PCR reactions contained 20 ng of template gDNA and 0.4 U of Q5 DNA polymerase (NEB) as well as a final concentration of 200 uM dNTPs, 2 mM MgCl2, and 0.5 uM of each primer. PCR reactions were thermocycled using the following protocol: 98 deg C for 30 sec, and 28 cycles at 98 deg C for 10 sec, 55 deg C for 30 sec and 72 deg C for 30 sec, with a final extension at 72 deg C for 2 min. Triplicate reactions per sample were pooled and gel-purified. In total, 151 libraries were paired end (2x 250bp) sequenced on the MiSeq (Illumina) at Duke's Genome Sequencing and Analysis Core Facility.

This data set and associated analysis is fully described in Yung et al. (2016) and Ward et al. (2017)

Data Processing Description

Sequences were demultiplexed and assigned to corresponding samples using CASAVA (Illumina) and deposited to NCBI Sequence Read Archive under accession number SRP068349.

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Data Files

File
accessions.csv (Comma Separated Values (.csv), 288 bytes) MD5:e2dd815c0b19aa682fb921f0586ce3c9 Primary data file for dataset ID 704695

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Related Publications

Hugoni, M., Taib, N., Debroas, D., Domaizon, I., Jouan Dufournel, I., Bronner, G., ... Galand, P. E. (2013). Structure of the rare archaeal biosphere and seasonal dynamics of active ecotypes in surface coastal waters. Proceedings of the National Academy of Sciences, 110(15), 6004–6009. doi:10.1073/pnas.1216863110
Methods

Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., & Schloss, P. D. (2013). Development of a Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq Illumina Sequencing Platform. Applied and Environmental Microbiology, 79(17), 5112–5120. doi:10.1128/aem.01043-13
Methods

Massana R, Murray AE, Preston CM, DeLong EF (1997). Vertical distribution and phylogenetic characterization of marine planktonic Archaea in the Santa Barbara Channel. Applied and Environmental Microbiology 63: 50-56.
Methods

Ward, C. S., Yung, C.-M., Davis, K. M., Blinebry, S. K., Williams, T. C., Johnson, Z. I., & Hunt, D. E. (2017). Annual community patterns are driven by seasonal switching between closely related marine bacteria. The ISME Journal, 11(6), 1412–1422. doi:10.1038/ismej.2017.4
Methods
,
Results

Yung, C.-M., Ward, C. S., Davis, K. M., Johnson, Z. I., & Hunt, D. E. (2016). Insensitivity of Diverse and Temporally Variable Particle-Associated Microbial Communities to Bulk Seawater Environmental Parameters. Applied and Environmental Microbiology, 82(11), 3431–3437. doi:10.1128/aem.00395-16
Methods
,
Results

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Parameters

Parameter	Description	Units
ID_number	NCBI ID number	unitless
ID_type	Type of NCBI ID number	unitless
NCBI_link	Link to NCBI metadata	unitless

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Instruments

Dataset-specific Instrument Name	Thermocyler
Generic Instrument Name	Thermal Cycler
Dataset-specific Description	Used for PCR reactions
Generic Instrument Description	A thermal cycler or "thermocycler" is a general term for a type of laboratory apparatus, commonly used for performing polymerase chain reaction (PCR), that is capable of repeatedly altering and maintaining specific temperatures for defined periods of time. The device has a thermal block with holes where tubes with the PCR reaction mixtures can be inserted. The cycler then raises and lowers the temperature of the block in discrete, pre-programmed steps. They can also be used to facilitate other temperature-sensitive reactions, including restriction enzyme digestion or rapid diagnostics. (adapted from http://serc.carleton.edu/microbelife/research_methods/genomics/pcr.html)

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Deployments

PICO_1-301

Website	https://www.bco-dmo.org/deployment/59063
Platform	Duke University Marine Lab
Start Date	2010-06-28
End Date	2012-06-26
Description	The PICO time series is sampled weekly (or more frequently) to capture physical, chemical and biological variability in the coastal ocean. This time series enables the investigator to collaborate with a number of researchers and will serve as a long-term research focus. Project information: http://oceanography.ml.duke.edu/johnson/research/pico/

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Project Information

Pivers Island Coastal Observatory (PICO)

Website: http://oceanography.ml.duke.edu/johnson/research/pico/

Coverage: 34.7181 deg N, 76.6707 deg W

From the project website:
Carbon dioxide is rising at ~3% per year in the atmosphere and oceans leading to increases in dissolved inorganic carbon and a reduction in pH. This trend is expected to continue for the foreseeable future and ocean pH is predicted to decrease substantially making the ocean more acidic, potentially affecting the marine ecosystem. However, coastal estuaries are highly dynamic systems that often experience dramatic changes in environmental variables over short periods of times. In this study, the investigators are measuring key variables of the marine carbon system along with other potential forcing variables and characteristics of the ecosystem that may be affected by these pH changes. The goal of this project is to determine the time-scales and magnitude of natural variability that will be superimposed on any long term trends in ocean chemistry.

This project is associated with Ocean Acidification: microbes as sentinels of adaptive responses to multiple stressors: contrasting estuarine and open ocean environments.

Collaborative Research: Ocean Acidification: microbes as sentinels of adaptive responses to multiple stressors: contrasting estuarine and open ocean environments (OA microbe adaptation)

Coverage: Neuse-Pamlico Sound to the Sargasso Sea

Extracted from the NSF award abstract:

This collaborative project by Duke University and Georgia Institute of Technology researchers will combine oceanographic and advanced molecular techniques to characterize the adaptive responses of microbial communities to multiple stressors associated with OA. In particular, microbial communities from estuarine and coastal ecosystems as well as open ocean waters will be incubated under conditions of increased acidity or temperature or both, and their activities will be measured and quantified.

Preliminary data from time-series observations of a coastal temperate estuary shows that pH, temperature and other stressors vary over multiple space and time scales, and this variability is relatively higher than that observed in open ocean waters. Based on this evidence, the guiding hypothesis of this work is that microbes in coastal ecosystems are better adapted to ocean acidification as well as multiple stressors compared to similar microbes from the open ocean. To quantify the adaptive genetic, physiological and biogeochemical responses of microbes to OA, the team's specific goals are to: (1) characterize complex natural microbial community responses to multiple stressors using factorial mesocosm manipulations, (2) assemble a detailed view of genomic and physiological (including transcriptional) adaptations to OA at the single species level using cultured model marine microbes (e.g. Prochlorococcus, Synechococcus, Vibrio) identified as responsive to stressors in whole community mesocosm experiments, and (3) assess the power of model microbial strains and mesocosm experiments to predict microbial community responses to natural OA variability in a temporally dynamic, temperate estuary and along a trophic/pH gradient from the Neuse-Pamlico Sound to the Sargasso Sea. By comparing an estuarine ecosystem to its open ocean counterpart, this study will assess the sensitivity of microbial structure and function in response to ocean acidification.

This project is associated with Pivers Island Coastal Observatory.

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Program Information

Science, Engineering and Education for Sustainability NSF-Wide Investment (SEES): Ocean Acidification (formerly CRI-OA) (SEES-OA)

Website: https://www.nsf.gov/funding/pgm_summ.jsp?pims_id=503477

Coverage: global

NSF Climate Research Investment (CRI) activities that were initiated in 2010 are now included under Science, Engineering and Education for Sustainability NSF-Wide Investment (SEES). SEES is a portfolio of activities that highlights NSF's unique role in helping society address the challenge(s) of achieving sustainability. Detailed information about the SEES program is available from NSF (https://www.nsf.gov/funding/pgm_summ.jsp?pims_id=504707).

In recognition of the need for basic research concerning the nature, extent and impact of ocean acidification on oceanic environments in the past, present and future, the goal of the SEES: OA program is to understand (a) the chemistry and physical chemistry of ocean acidification; (b) how ocean acidification interacts with processes at the organismal level; and (c) how the earth system history informs our understanding of the effects of ocean acidification on the present day and future ocean.

Solicitations issued under this program:
NSF 10-530, FY 2010-FY2011
NSF 12-500, FY 2012
NSF 12-600, FY 2013
NSF 13-586, FY 2014
NSF 13-586 was the final solicitation that will be released for this program.

PI Meetings:
1st U.S. Ocean Acidification PI Meeting(March 22-24, 2011, Woods Hole, MA)
2nd U.S. Ocean Acidification PI Meeting(Sept. 18-20, 2013, Washington, DC)
3rd U.S. Ocean Acidification PI Meeting (June 9-11, 2015, Woods Hole, MA – Tentative)

NSF media releases for the Ocean Acidification Program:

Press Release 10-186 NSF Awards Grants to Study Effects of Ocean Acidification

Discovery Blue Mussels "Hang On" Along Rocky Shores: For How Long?

Discovery nsf.gov - National Science Foundation (NSF) Discoveries - Trouble in Paradise: Ocean Acidification This Way Comes - US National Science Foundation (NSF)

Press Release 12-179 nsf.gov - National Science Foundation (NSF) News - Ocean Acidification: Finding New Answers Through National Science Foundation Research Grants - US National Science Foundation (NSF)

Press Release 13-102 World Oceans Month Brings Mixed News for Oysters

Press Release 13-108 nsf.gov - National Science Foundation (NSF) News - Natural Underwater Springs Show How Coral Reefs Respond to Ocean Acidification - US National Science Foundation (NSF)

Press Release 13-148 Ocean acidification: Making new discoveries through National Science Foundation research grants

Press Release 13-148 - Video nsf.gov - News - Video - NSF Ocean Sciences Division Director David Conover answers questions about ocean acidification. - US National Science Foundation (NSF)

Press Release 14-010 nsf.gov - National Science Foundation (NSF) News - Palau's coral reefs surprisingly resistant to ocean acidification - US National Science Foundation (NSF)

Press Release 14-116 nsf.gov - National Science Foundation (NSF) News - Ocean Acidification: NSF awards $11.4 million in new grants to study effects on marine ecosystems - US National Science Foundation (NSF)

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1416665

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